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Cellular Localization and Functional Properties of Nicotinic Receptors on Cultured Cortical Neurons from Rat Brain.

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Our laboratory previously identified putative nicotinic receptor sites in primary monolayer cultures of cortical neurons isolated from fetal rat brain (*J. Pharmacol. Exp. Ther.* 246: 409-416 (1988)). The equilibrium and kinetic binding properties of these sites, as well as their pharmacological specificity, are essentially the same as those determined for high affinity receptors in adult rat brain tissue. Methods have now been developed to localize these sites at the cellular level and to define their functional properties. We have found that anti-idiotypic antibodies against anti-nicotine antibodies are potent competitive inhibitors of [3 H]-nicotine binding to high affinity sites on the cells. These antibodies represent structural analogs of nicotine which have been shown to recognize neuronal nicotinic receptors (Bjercke, R.J. and J.J. Langone, *Biochem. Biophys. Res. Commun.* 162: 1085-92 (1989)). Consequently, we have used them to identify and characterize populations of receptor-bearing neurons by means of indirect immunofluorescence. We also have begun to explore the functionality of these receptor sites, using the ion-specific fluorescent probe fura-2 in conjunction with light microscopic image analysis techniques to measure ligand-evoked changes in intracellular calcium. The results indicate that the nicotine binding sites associated with these cells are functional nicotinic cholinergic receptors.

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